FINAL REPORT

Project title:
Nodulation and Rhizobial Specificity of *Amorpha nitens* in two Illinois Populations

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I. Introduction.

This is the final report of the first phase of a nodulation study of the native legume *Amorpha nitens* (smooth false indigo) to be presented to the Illinois Department of Natural Resources. Several experiments were performed to determine if *A. nitens* is nodulated by nitrogen fixing rhizobia bacteria (effectiveness) and if rhizobia bacteria affect the growth (efficiency) of *A. nitens* seedlings. Initially rhizobia bacteria were extracted from nodules of *A. nitens* collected from approximately 10 plants from two sites. Forty pure rhizobia strains were identified and characterized. The first two experiments established during the proposed timeframe of this grant failed due to uncontrollable causes including herbicide drift and very high white fly infestation in the greenhouse. Other experiments were established during 1998 and terminated in November 1998. Three strains were randomly selected and used to inoculate *A. nitens* seedlings in the greenhouse. Also symbiotic promiscuity or compatibility of four *A. nitens* strains was tested on seedlings of *A. canescens*, *A. nana*, *A. fruticosa*, *and Baptisia leucantha.*

The results of the experiments confirm the hypotheses that *A. nitens* is symbiotic to rhizobia bacteria and are compatible with *A. nana*, *A. canescens*, and *A. fruticosa* but not on *Baptisia leucantha*. Nodulation was significantly different on seedlings of *A. nitens*, *A. nana, A. canescens*, and *A. fruticosa* inoculated with the different rhizobia. Nodulation affected root length on *A. nitens* and shoot length of *A. canescens.* Rhizobia bacteria were re-isolated from nodules of *A nitens* seedlings to complete Koch's postulates.

Additional experiments are being done under a highly controlled white fly-free environment within the USDA Forest Service laboratories at the University of Missouri at Columbia to test additional rhizobia strains.

II. Literature Review

Amorpha nitens Boynton (smooth or shining false indigo) is an endangered plant in Illinois (Herkert 1991). Only three populations were known to occur in Pope county in southern Illinois (Taft 1994); however, other populations are likely to exist in adjacent areas (Jody and Beth Shimp, personal communication). The natural range of *A. nitens* extends from Georgia to Arkansas, north to southern Illinois (Fernald 1970, Herkert 1991); however, the number of occurrences are only listed in Illinois (Ostlie 1994). *Amorpha nitens* is poorly documented in other states within the range. Plant reproduction of *A. nitens* is poor within southern Illinois (Taft 1994). Initially, winter freeze damage and lack of suitable habitat were considered the main causes of its limited numbers. Other threats include invasion of exotic species such as *Lonicera japonica* and hydrological perturbations caused mainly by dam construction (Ostlie 1994).

Amorpha nitens is a legume that belongs to the Papilionoideae subfamily. It is a perennial shrub 1 to 3 m in height, usually glabrous. It has 9 to 19 oblong or oblong-ovate leaflets. Flowers have only one purple petal (the standard). Flowers are produced from May to June. In late August, mature pods produce 1 to 2 seeds. Fruits may remain on the plant during winter (Mohlenbrock 1959, Polhill and Raven 1978, Ostlie 1994, Taft 1994). Three Illinois populations grow in sites with strongly to extremely acid soils.

There are from 20 to 25 *Amorpha* species (Allen and Allen 1981). All species are

indigenous to North America, from southern Canada to Mexico. *Amorpha nitens* and *A. fruticosa* are found in thickets and banks of streams and may share the same sites. These two species are easily confused in the field, except that *A. nitens* leaves become dark after drying and those of *A. fruticosa* remain green (Gleason and Cronquist 1991, Beth Shimp, personal communication). Both, *A. canescens* and *A. nana* are found in prairies and plains (Britton 1970). *Amorpha canescens* and *A. fruticosa* are propagated by seeds or cuttings. Hartman et al. (1997) suggest to acid-scarified the seeds for 10 to 15 minutes followed by 2 to 8 weeks of stratification. Recently, seeds of *A. nitens* germinated readily without any scarification treatment (John Taft, personal communication).

Although reports of rhizobia bacteria nodulating *A. nitens* have not been documented, nodulation has been observed in mature plants (J. Taft, personal communication). *Amorpha canescens* and *A. fruticosa* and 5 other species are reported to be nodulated by rhizobia bacteria (Allen and Allen 1981). *Amorpha nitens* and *A. nana* are not reported. Cross-inoculation studies with rhizobia bacteria from *Amorpha* and other legumes suggest the presence of a specific rhizobia-plant group for *Amorpha.*

III. Materials and Methods

Part I

1. Field Studies. Site description.

Previous to the submission of the proposal only two populations had been reported to exist for *A. nitens.* During the period of the study 4 other populations were reported (Jody Shimp, personal communication). For this study three populations were surveyed to locate *A. nitens* populations and associated vegetation. These three sites are found near road 146 along Big Grand Pierre Creek, 5 miles north of Golconda, Illinois (See proposal). Site 1 is located both on USDA Forest Service land and private land, and sites 2 and 3 are located on private property owned by Joan and Bob Perry.

Site 1 is located on both sides of IL 146 road along Big Grand Pierre Creek. Site 2 is found in a steep area along Horseshoe Lake and site 3 is mostly a flat area that gets flooded regularly by the same lake. This site is divided by an opening created by the Electricity Company that runs a power line through the Perrys' land.

During the visits previous to grant approval, roots of plants from site 1 were examined and nodules were collected for initial strain isolations at Southern Illinois University. After grant approval, more visits were done to locate sites 2 and 3 (See proposal).

2. Soil, nodule and seed collection.

Following Halliday (1991) and Navarrete-Tindall and Van Sambeek (1996) guidelines, six to ten plants per site were studied for the presence of nodules. If present, nodules were collected and stored in airtight containers containing silica gel. Plant data recorded included height in cm, basal stem diameter in mm, number of basal stems and branches per stem, evidence of old growth by searching for underground stem, presence of insects or fungi, presence of flowers or fruits, and associated vegetation. Also one leaf per plant was collected and dry pressed for correct plant identification. *A. nitens* foliage turns dark after drying compare to *A. fruticosa*, which remains green (Jody and Beth Shimp, personal communication). Soil samples were collected from under each plant and a composite sample for each site was made. Samples of potting soil and garden soil were also included. Nutrient analyses included % organic matter (OM), lbs. per acre of phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg), and soil pH. Alvey Labs in Illinois performed analyses. Presence of micronutrients was also analyzed but it is not included in this report.

3. Nodule characterization

Fresh nodules collected in the field were studied in the laboratory. Data recorded

included shape, size, and internal and external color[1]. Nodules were classified using Corby (1988). The presence of red, brown or pink internal color indicates the presence of leghemoglobin from efficient bacteria. Also a smear from some nodules was prepared using carbolfuchsin solution to observe the presence of bacteroids inside nodules. Bacteroids are rhizobia bacteria that become pleomorphic (different irregular shape) inside the nodules. Randy Tindall preserved several nodules in glyceraldehyde for further scanning electron microscope studies.

4. Isolation, characterization, and identification of rhizobia bacteria

Following procedures outlined by Navarrete-Tindall (1996), nodules with pink to red centers were chosen for rhizobia isolation. Ten nodules per plant (6 to 10 plants per site) were randomly selected and only two or three nodules per plant were used to isolate rhizobia bacteria. Initially nodules were rehydrated in sterile distilled water at room temperature for 1 hour or at 5°C overnight. Nodules were sterilized by placing them in 95% ethanol from 10 to 15 seconds, transferred to 3% hydrogen peroxide (H_2O_2) for 2 to 3 minutes, and washed twice in sterile distilled water.

Each nodule was crushed with a glass rod inside a sterile culture tube with 0.1 ml distilled water. Part of the solution was inoculated on petri dishes containing yeast mannitol agar (YMA) medium with 25 ppm crystal violet. Ten days later, two to three individual colonies (each colony is considered to be a strain) were transferred into 2 petri dishes containing YMA with 25 ppm congo red. Four individual, non-stained, round colonies per nodule were randomly selected for a total of 24 to 40 strains per site. Each selected strain was characterized with the gram stain technique to assure that they were gram-negative bacteria (gram-negative bacteria stain pink and gram-positive stain purple). Strains were transferred to four petri dishes containing peptone glucose agar (PGA) media. Rhizobia strains do not grow well on PGA. This test was done to reassure

[1] These results will be included in a paper to Agroforestry Systems.

that bacteria isolated were rhizobia strains.

Twelve strains were characterized by inoculating 4 petri dishes each containing YMA with congo red. One strain isolated from the tree legume *Pithecellobium saman* was also inoculated on the same medium. Data recorded at day 3 or 4, and day 10 included colony shape, colony size, presence of gum, number of colonies, and color of colonies[1]. All strains were transferred to culture tubes containing YMA-only and stored at 5°C. These pure strains were used to prepare yeast mannitol broth cultures to inoculate seedlings.

5. Seed Germination

All seeds were sterilized for 1 minute in 95 % ethanol, 3 to 5 minutes in 3 % hydrogen peroxide, and washed twice in sterile distilled water. Sterile seeds were moist-stratified at 5 °C for 10 days. Before germination seeds were planted in containers with moist vermiculite or Sunshine Mix® medium or left in petri dishes. Both containers and petri dishes with seeds were placed under fluorescent light in the greenhouses or laboratory. Seeds left in petri dishes were flooded with sterile distilled water.

Part 2.

Rhizobia efficiency and effectiveness on four *Amorpha* spp.

1. Establishment of greenhouse experiments.

Seedlings growing in pots were watered once every 7 to 10 days, once with sterile water and next time with full or half-strength Broughton and Dilwort's medium (Somasegaran and Hoben 1994). Seedlings were inoculated twice with 1.5 ml of the broth cultures inoculated with the selected strains. The first inoculation was done 5 days after potting the seedlings and the second inoculation was done 15 days after the first inoculation.

Data recorded included: root length, epicotyl length, number of nodes, shoot or root diameter, and number, size, shape, and weight of nodules.

2. Data collection and analyses

For most experiments, quantitative measurements were made after seedling roots were washed free of potting medium. All experimental designs were completely randomized. Data were analyzed with the Windows version of the Statistical Analyses System (SAS Institute Inc.). Analyses of variance was done using PROC GLM to test for significance among treatments at the p=<0.05 (*) and p=>0.05 (ns). When significant differences occurred, treatment means were compared using Fisher's Least Significance Difference test (LSD) at the p=<0.05(*).

3. Experiments

Five experiments were performed involving rhizobia bacteria isolated from *A. nitens* plants and their ability to nodulate *A. nitens* and other native legumes. Experiments 1 and 2 were performed to investigate the ability of several rhizobia strains to nodulate (effectiveness) and affect growth (efficiency) of *A. nitens*. In addition, experiments 3, 4, and 5 were conducted to investigate the ability of several *A. nitens* strains to nodulate and affect growth of *A. nana, A. canescens, A. fruticosa*, and *Baptisia leucantha*.

Experiment 1. Run 1 and 2. Effect of rhizobia strains of *A. nitens* on seedlings of *A. nitens*, *A. fruticosa*, *A. nana*, and *Baptisia leucantha*. Seedlings were inoculated with six *A. nitens* strains (AN4, AN6, AN25, AN26, AN101, AN111), one strain from *Pithecellobium saman*, and with YMB-only, (these last two treatments as controls) to test the effect on seedling growth. Each treatment consisted of 10 seedlings grown individually in deepots® containing vermiculite. This experiment was not completed due to herbicide drift of the first run and high white-fly infestation of the second run. Data

were not recorded due to bad seedling development.

Experiment 2. Effect of rhizobia bacteria from *A. nitens* on growth of seedlings of *A. nitens*. The effect of three *A. nitens* strains was tested on *A. nitens* growth. Four treatments included inoculation with AN26, AN101, and AN111 and with YMB-only. The treatments consisted of 8 seedlings planted individually in pots 10 cm in diameter and 15 cm in height containing Sunshine Mix®. Number of seedlings per treatment was 8. The experiment was terminated 90 days after the first inoculation was done.

Experiment 3. Symbiotic promiscuity of rhizobia strains from *A. nitens* on seedlings of *A. canescens*. Seedlings of *A. canescens* were inoculated with strains AN25, AN101, AN111, and with YMB-only as control. The purpose of this experiment was to evaluate the compatibility of rhizobia (cross-inoculation) strains and their effect on *A. canescens* seedling growth. Eight seedlings per treatment were grown in 10x8x15 cm pots containing vermiculite. The experiment was terminated 80 days after the first inoculation.

Experiment 4. Symbiotic promiscuity of rhizobia strains from *A. nitens* on seedlings of *A. nana*. *Amorpha nana* seedlings were inoculated with strains AN26, AN101, AN111, and YMB-only. The purpose of this experiment was to evaluate the compatibility of rhizobia strains and their effect on *A. canescens* seedling growth.
Eight seedlings per treatment were grown in 10x8x15 cm pots containing vermiculite.
The experiment was terminated 90 days after the first inoculation.

Experiment 5. Symbiotic promiscuity of rhizobia strains from *A. nitens* on seedlings of *Amorpha fruticosa* and *Baptisia leucantha*. The strains AN25 and AN101 were inoculated on seedlings of *Amorpha fruticosa* and *Baptisia leucantha* growing in 10x8x15 cm pots containing vermiculite and the strain AN101 was inoculated on *Amorpha*

fruticosa seedlings growing in pots containing Metro Mix. These plants were harvested 100 days after first inoculation. No data analyses were done because no experimental design was established.

IV. Results

1. Site description

Site 1: Woodland community, some of the associated vegetation include *Quercus marilandica* and other *Quercus* species, *Carya* sp., *Cornus florida*, *Sassafras albidum*, *Lindera benzoin*, *Toxicodendron radicans*, *Symphoricarpos orbiculatus*, *Wisteria*, *Trifolium sp.*, *Avena sp.* and others. *A. nitens* was abundant next to the creek near the road in an area of 25 m^2 and very scarce in the rest of the studied area, which was approximately 40 m long and 10 m wide. This is a very disturbed site due to human impact and the presence of road 146.

Site 2: *A. nitens* plants were located in the lower part of a flatland with limestone and fragipan layer (Jody Shimp, personal communication). Water remains after a rain and during hot days the land becomes very dry, so we observed plants belonging to lowlands and open lands. The upper part is very steep and suddenly becomes less steep when we get close to the lake, which is the result of damming the Grand Pierre Creek.
The understory vegetation was scattered in the steep areas. The understory vegetation was dense about 10 m from the shore. Some associated vegetation includes *Quercus muehlenbergii*, *Q. shymardii*, *Acer saccharum*, *Juglans nigra*, *Asclepias* sp., several *Carex* spp., *Senna* sp., *Baptisia leucantha*, and *Desmodium pocaflora*. Other vegetation found in this same site includes *Ruella* sp., *Campanula* sp., *Avena* sp., *Symphoricarpos orbiculatus*, *Commelina virginiana*, *Ulmus* sp., *Robinia pseudoacacia* seedlings, *Bambusa* sp. and possibly *A. fruticosa*.

A. nitens plants found were 1 to 10 m from the shore. Plants closer to the shore

receiving more light, had fruits, and seemed to have more nodules. Next to this site in an area 150 m long adjacent to the shore of the lake only two *A. nitens* plants were found. *Bambusa* sp. dominated this area.

Site 3. This site is also located on Joan and Bob Perry's land and is found at the banks of Horseshoe Lake. This area is regularly flooded for short periods and understory vegetation was scarce. There is an opening created by the Electricity Company that runs a power line through the Perrys' land. This opening creates a different habitat for *A. nitens* that in the other two sites mentioned above. *Amorpha nitens* studied in this site were taller and had more fruits than in the above 2 sites. Some of the understory vegetation included grasses, herbaceous legumes as well as various tree seedlings.

2. Soil Nutrient Analyses

Nutrient analyses showed variation for soils collected from 3 sites, potting medium, and garden soil (Table 1). The amount of P was much higher for greenhouse soil than for soil from the other 4 locations. Organic matter was much lower for garden soil than for the other 4 locations. K, Ca, and Mg amounts were low or medium for all soils in crop production.

Table 1. Soil nutrient analyses for soils collected from three *Amorpha nitens*, potting soil, and garden soil used to grow *A. nitens* plants.

Location[b]	Soil pH	P[a]	K[a]	Ca[a]	Mg[a]	% OM
Site 1	4.9	26	250	1955	485	4.6
Site 2	5.4	13	208	3063	483	4.3
Site 3	5.6	14	175	2980	285	5.2
Potting soil[c]	5.8	92	186	3290	470	6.0
Garden TIC[c]	6.6	43	153	3965	345	1.7

a/ lbs. per acre, b/ Site 1, 2, and 3, were previously described. Potted soil used in the greenhouse to grow several A. nitens plants, c/ Tree Improvement Center at Carbondale, Illinois.
Values are the average of 2 or 3 samples. Analyses were done at Alvey Laboratory, Carlyle, IL.

3. Germination Studies

Stratified seeds of *Amorpha nitens*, *A. nana*, *A. canescens*, and *A. fruticosa* were planted in vermiculite or left in petri dishes with distilled water under fluorescent light for germination studies. All four species were propagated from seeds stratified for 10 days at 5°C in sterile-moist conditions. Fifteen-days after stratification, 86% of the *A. nitens* seeds exposed to light germinated in petri dishes and only 40% of the seeds germinated in pots filled with vermiculite. Between 95% and 100% of the seeds of *A. nana, A. canescens* and *A. fruticosa* germinated in petri dishes exposed to light 9 days after stratification. In further studies, 100% of non-stratified seeds of *A. nana* germinated at day 3 after sowing in moist vermiculite.

4. Rhizobia efficiency and effectiveness on 4 *Amorpha* species.

Experiment 1. Run 1 and 2. Effect of 3 rhizobia strains from *A. nitens* on seedlings of *A. nitens*, *A. fruticosa*, *A. nana*, and *Baptisia leucantha*. These two experiments were terminated due to herbicide drift and high whitefly infestation. Plants did not develop

sufficiently to record any useful data.

Experiment 2. Effect of 3 rhizobia strains from *A. nitens* on growth of seedlings of *A. nitens*. The three rhizobia strains AN26, AN101, and AN111 produced nodules on *A. nitens* seedlings. AN26 and AN101 produced nodules on 88% of the plants and AN111 produced nodules on 100% of the plants. No nodules were observed on non-inoculated seedlings (Table 2). Nodule number was significantly different between seedlings inoculated with one of the three strains and seedlings inoculated with YMB-only. Number of nodules for inoculated plants varied from 35 to 56 per plant. Epicotyl length was significantly different for seedlings inoculated with AN26 and AN111 and the control YMB-only but not between seedlings inoculated with strain AN101 or YMB-only. No significant differences were observed for nodule weight, node number, and root length.

Experiment 3. Effectiveness and efficiency of 3 rhizobia strains from *A. nitens* on seedlings of *A. nana*. All three rhizobia strains of *A. nitens* produced nodulation on *A. nana* seedlings. Strains AN26, AN101, and AN111 nodulated 88%, 78%, and 13% of the seedlings, respectively. Seedlings inoculated with YMB-only produced no nodules (Table 3). Nodule number was significantly different among treatments. Nodule number was higher for seedlings inoculated with AN26 and AN101 strains than for seedlings.

Table 2. Effect on growth on *Amorpha nitens* seedlings 90 days after inoculation with rhizobia bacteria from *A. nitens.*

Rhizobia strain	Nodulation[a] (%)	Nodule number[a]	Nodule weight[a] (g)	Hypocotyl length[a] (cm)	Root length[a] (cm)	Node number[a]
AN26	88	35	0.105	7.5	14.1	12.4
AN101	88	35	0.110	5.5	8.3	12.9
AN111	100	56	0.173	7.5	8.8	13.2
Control	0	0	0.0	4.8	8.2	12.4
Sign.[B]	n.a	**	n.s	n.s	**	n.s

[A]Each value represents the mean of 8 seedlings with and without nodules.
[B]Significant main effect at 1% level according to the F-test.

inoculated with strain AN111 or YMB-only. No significant differences were observed for epicotyl, hypocotyl, and root length or node number.

Experiment 4. Effectiveness and efficiency of 3 rhizobia strains from *A. nitens* on seedlings of *A. canescens.* All three rhizobia strains of *A. nitens* produced nodulation on *A. canescens* seedlings. Percent nodulation was higher for seedlings inoculated with AN101 and AN111 strains than for seedlings inoculated with strain AN25. YMB-only produced no nodules (Table 4). Nodule number, epicotyl length, and root diameter were significantly different among treatments. No significant differences were observed for hypocotyl and root length.

Table 3. Effect on growth of *Amorpha nana* seedlings inoculated with 3 rhizobia strains of *A. nitens.*

Rhizobia strain	Nodulation[a] (%)	Nodule[a] no.	Epicotyl length[a] (cm)	Hypocotyl length[a] (cm)	Root length[a] (cm)	Node[a] no.
AN26	88	3.8	4.02	1.15	7.78	9
AN101	78	2.7	3.99	1.03	6.36	7
AN111	13	0.1	3.84	1.07	5.83	8
YMB-only	0	0	4.32	1.20	9.56	8
Sign.[b]	n.a	**	n.s	n.s	n.s	n.s

[a]Each value represents the mean of 8 seedlings with and without nodules.
[b]Significant main effect at 1% level according to the F-test, 3 and 29 degrees of freedom.

Experiment 5. Effectiveness and efficiency of 2 rhizobia strains from *A. nitens* on seedlings of *Amorpha fruticosa* and *Baptisia leucantha* grown in different growing media. Strains AN25 and AN101 produced nodules on *A. fruticosa* seedlings but not on *B. leucantha* seedlings. AN101 produced more nodules and better developed epicotyls and roots on *A. fruticosa* seedlings than AN25. Plants inoculated with AN101 growing in different media show some variation as well. Seedlings grown in Metro-mix had more nodules and better developed epicotyls and roots than seedlings grown in vermiculite.

Table 4. Effect on growth of *Amorpha canescens* seedlings inoculated with 3 rhizobia strains of *Amorpha nitens.*

Rhizobia strain	Nodulation[a] (%)	Nodule[a] no.	Epicotyl length[a] (cm)	Hypocotyl length[a] (cm)	Root length[a] (cm)	Root[a] dia. (mm)
AN25	22	0.3	5.6	1.0	8.9	1.4
AN101	88	16	4.6	0.7	8.1	1.3
AN111	100	14	5.6	1.4	7.8	1.4
YMB-only	0	0	3.2	1.0	7.2	0.9
Sign.[b]	n.a	**	**	n.s	n.s	**

[a]Each value represents the mean of 8 seedlings with and without nodules.
[b]Significant main effect at 1% level according to the F-test, 3 and 29 degrees of freedom.

Table 5. Effect on growth of *Amorpha fruticosa* inoculated with 2 rhizobia strains of *Amorpha nitens*

Rhizobia strain	Growing medium	Nodulation[a] (%)	Nodule[a] no.	Epicotyl length[a] (cm)	Hypocotyl length[a] (cm)	Root length[a] (cm)
AN25	Metro Mix	43	1	4.0	1.3	4.3
AN101	Metro Mix	89	33	7.1	1.1	12.1
AN101	Vermiculite	62	8	6.5	1.1	8.8

[a]Each value represents the mean of 8 seedlings with and without nodules.
These values can not be analyzed because no control was included in experiment

Other studies.

We planted two 6-month old containerized seedlings in May of 1997 in a garden (see Table 1 for soil nutrient analyses) exposed to full sunlight at the Tree Improvement Center in Carbondale. Both plants bloomed in August 1997 and 1998. Seed production was high. Seeds from these two plants were used for germination studies. Plants have been cleaned of weeds and watered during dry periods. Plants are 1 m apart from each other and 2 m apart from other woody plants so competition for light and soil nutrients is

minimal. One more containerized plant was planted in Spring 1998 in shady conditions on adjacent 10-year old close spaced mixed hardwood stand. Four more plants were maintained in the greenhouse, one bloomed in July 97 and produced fruits also. The second run of experiments 2, 3, 4, and 5 are established in the USDA Forest Service Laboratories at the University of Missouri in Columbia. Surviving seedlings from these studies will be outplanted at the Horticulture and Agroforestry Center (HARC) in New Franklin, Missouri.

Conclusions

1. Soil pH and macronutrient concentrations on the 3 sites indicate that *A. nitens* grow well in acidic conditions as previously reported by Taft (1994), and low and medium amounts of P, K, Ca, and Mg (See table 1).
2. Plants growing in the garden and in the greenhouse at the Tree Improvement Center in Carbondale fruited the first year in the field. This may indicate that *A. nitens* is adapted to a wide range of soil P and organic matter content. This suggests that A. nitens may grow in other areas if water is available.
3. Bacteria isolates from *A. nitens* were shown to be rhizobia by producing nodules on seedlings of *A. nitens*. The Koch's postulates were completed after reisolation of rhizobia bacteria from seedlings.
4. Almost all strains from *A. nitens* (AN25, AN26, AN101, and AN111) were effective (produced nodules) on *A. nitens*, *A. fruticosa*, *A. canescens*, and *A. nana*. Strain AN25 and AN111 were not effective on *A. canescens* and *A. nana* seedlings (Tables 2 to 5).
5. There was variation in nodulation on seedlings inoculated with the above strains (Tables 2 to 4). Nodulation was the highest for *A. nitens*, and *A. canescens* seedlings inoculated with strain AN111, while nodulation was the lowest for *A. nana*.

Strains AN111 and AN26 produced 78% to 89% nodulation on all species tested. Strain AN25 produced less than 50% nodulation on *A. canescens* and *A. fruticosa* seedlings.

6. None of the seedlings inoculated with YMB-only produced nodules. Significant differences were observed for the number of nodules per seedling (when inoculated with different strains from *A. nitens*) for *A. nitens, A. fruticosa, and A. canescens* seedlings. *Baptisia leucantha* seedlings inoculated with strains AN26, AN101, and AN111 did not cross-inoculate (did not produce nodules).
7. Significant differences were observed for root length of *A. nitens* inoculated with strain AN26. Root diameter and epicotyl length were significantly higher for *A. canescens* seedlings inoculated with strains AN25, AN101, and AN111than for seedlings inoculated with YMB-only.
8. Plants developed well when planted in well drained soil, under open conditions, with limited competition from associated vegetation, and watered regularly at the Tree Improvement Center in Carbondale.

Recommendations

Since *A. nitens* are nodulated with rhizobia bacteria, we are planning to grow *A. nitens* seedlings in soil collected from the three *A. nitens* sites which showed pronounced differences in *A. nitens* growth and health. The purpose is to evaluate plant growth by rhizobia bacteria present in these soils and different levels of shade.

Management is necessary to maintain or increase the number of *A. nitens* plants within existing sites and to establish new populations. This includes maintaining the sites open to semi-open and controlling the growth of exotic species.

Further studies

Presently we are evaluating a total of 15 *A. nitens* strains including the same strains already studied.

Evaluation of the effect of 4 levels of shade on containerized *A. nitens*, *A. fruticosa*, *A. canescens*, and *A. nana* seedlings.

Establishment of *A. nitens* seedlings in original sites in Pope county, Illinois, and other riparian sites at the Horticultural and Agroforestry Center in New Franklin Missouri, under different shade levels.

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Photo 2.
Donald Lawrence, Georgeann Hartzog, and Jerry Van Sambeek next to two A. nitens plants in site 3.

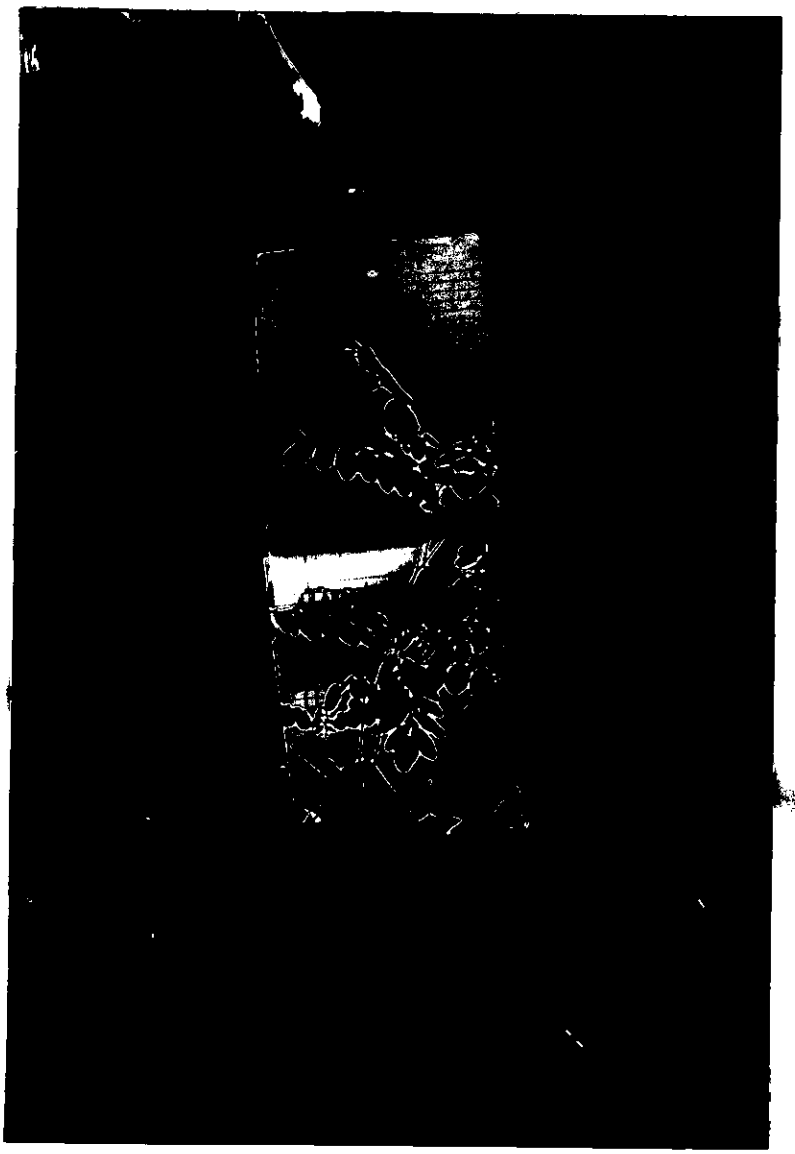

Photo 1.
Jerry Van Sambeek showing an Amorpha nitens plant found on site 1.

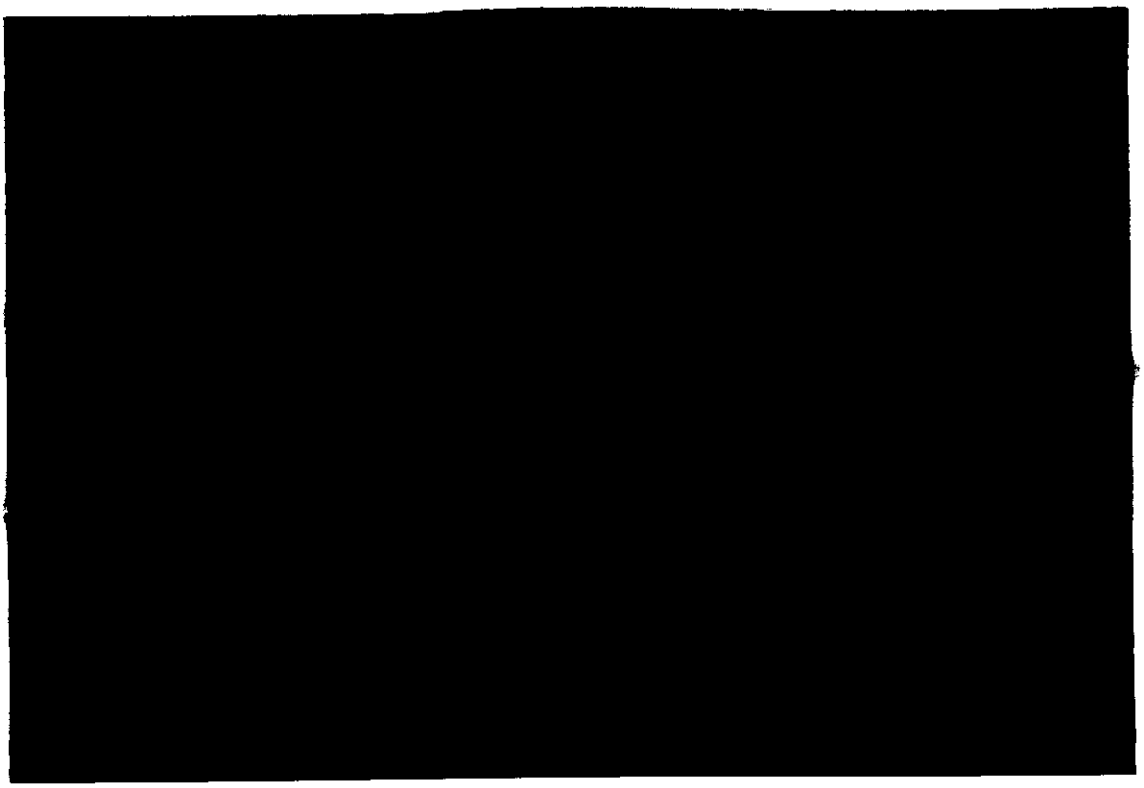

Photo 3. Root nodules collected from an *A. nitens* plant.
Nodule size is 2 to 3 mm.

Photo 4. Five-month old *Amorpha nitens* plant growing in the greenhouse with one inflorescence.

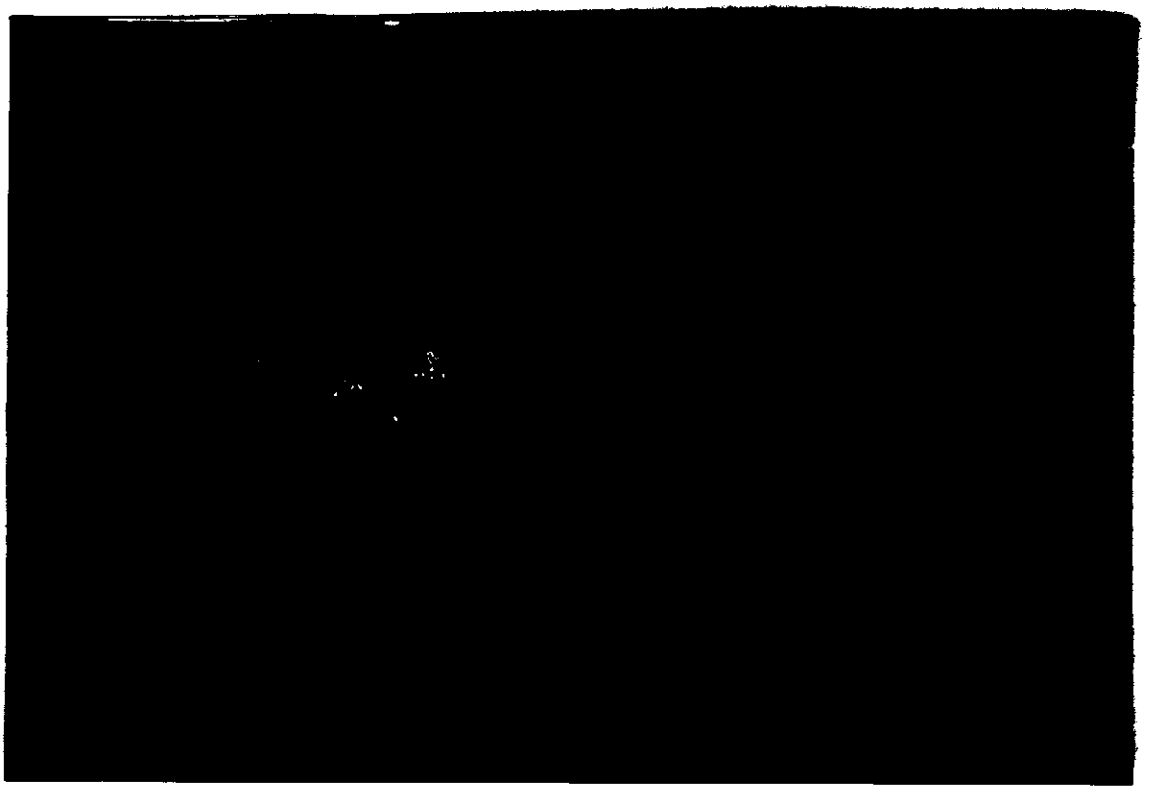

Photo 5. Close up of same inflorescence shown in photo 4.

Photo 6a. Two-month old Amorpha nitens seedling.
Photo 6b. Same plant five to six month-old growing in a garden at the Tree Improvement Center in Carbondale.